SIXTH QUARTERLY SUMMARY REPORT OF PROGRESS

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REDUCTION OF MICROBIAL DISSEMINATION

GERMICIDAL ACTIVITY OF ETHYLENE OXIDE

REDUCTION OF MICROBIAL CONTAMINATION ON SURFACES

by the

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Reduction of Microbial Contamination on Surfaces -- Three methods have been investigated for contaminating various surface materials for studies on the reduction of microorganisms held under environmental conditions of 50 C and 40% relative humidity. Procedures and results of two methods have previously been described: (1) contamination by artificial seeding (Third Quarterly Report), and (2) contamination by exposure to intramural air in areas occupied by humans (Fifth Quarterly Report).

In summary, *Salmonella derby*, *Escherichia coli*, and *Bacillus globigii* seeded onto various surface materials and subjected to the controlled environment showed high death rates during the first 1/2 hour, followed by slower rates of decrease during progressive exposure for periods up to 168 hours. With the second method, viable contamination was acquired slowly and non-uniformly on surface materials during prolonged periods of exposure to intramural air in areas with high or moderate human activity. Variations found between strips exposed in parallel produced standard errors (range: 0 to $1.1 \times 10^6/ft^2$) impracticable to reduce for death-rate analysis.

A method for obtaining maximum, evenly distributed, human-associated viable contamination on surfaces is necessary for accurate death-rate determinations. Further, it is important to minimize the natural selection inherent to viable contamination collected over extended periods. It is equally important to use sampling procedures designed for obtaining total counts of viable cells. Methods used should include techniques for the isolation and enumeration not only of non spore-forming organisms, but also species of Bacillus and Clostridia. These experimental criteria have not been easy to achieve collectively when large continuous surface areas were used for study.

A third study method was investigated for obtaining more precise control of the factors discussed above. Trays containing 60 sterile 1 x 2 inch strips of stainless steel were contaminated with human shed organisms during 1-hour periods of exposure in an occupied, plastic shedding chamber. The chamber was operated at slight positive pressure, with 1 air-volume change every 4.5 minutes. Input air to the chamber was through filters of 99.97 + % efficiency. Immediately after the exposure period, the strips were placed at random into covered, sterile 150 mm plastic Petri dishes using aseptic techniques. Five strips were placed into each of 10 plates and 10 strips were selected for assay and enumeration of the contaminating load. The plates containing strips to be exposed at 50 C and 40% R.H. were immediately placed onto a pre-adjusted, dynamic climatic chamber. The contaminating load was determined by placing each of the 10 selected strips into separate tubes containing trypticase soy broth. The cells were eluted from the strips by mechanical agitation for 15 minutes. The cell suspensions then were equally divided; one undiluted portion was immediately plated for growth of non spore-forming organisms, and the other undiluted portion was heat shocked at

80 C for 20 minutes before plating for the enumeration of spore-forming organisms. All samples were plated into 150 mm Petri plates and mixed with trypticase soy agar. Colonies were counted after incubation for 48 hours at 37 C and again after further incubation for 3 days at room temperature. Reduction of viable microorganisms on the strips held at 50 C and 40% R.H. were determined at pre-determined time intervals. For each timed assay, 10 strips were randomly selected; culture procedures were the same as those described above. Variations in microbial loads on strips exposed at the same time were greatly reduced using the described techniques for obtaining contamination. Aerobic spore counts were low in all samples examined; anaerobic spore counts were not determined for these initial experiments. Definitive identification of contaminating organisms was not performed. An increase of approximately 25 percent in the numerical colonial growth was observed after incubation at room temperature for 3 days. Colony counts were not difficult to assess because colonial spreading and coalescing were minimal in the large Petri plates. Reductions of about 90% or greater of viable cells on strips were achieved in most instances, during the first 24 hours at 50 C and 40% R.H. Results obtained are shown in table 1.

These data suggest that the methods used for contaminating surface materials and the laboratory assay techniques used may give more accurate and meaningful information than previous or proposed study methods on the effects of environmental conditions on viability of human-associated microorganisms. The recognized advantages for using the described method of study are summarized below:

1. Short exposure period to contamination.
2. Reduction of natural selection.
3. Reduced numbers of non-human associated microorganisms.
4. Reduced variation in contamination loads.
5. Quantitation of total viable cell counts.
6. Accurate assay for spores and anaerobic microorganisms is possible.

Reduction of Microbial Dissemination -- Studies on reduction of microbial dissemination from humans using the microbiotank were extended. Comparative data were obtained on microbial shedding by one male subject dressed in the following types of clothing: (1) clean-room "bunny suit", hood, and sterile socks; (2) clean-room coat, cap, sterile socks, and personal trousers; (3) personal street clothing and sterile socks. Prior to use, the clean-room clothing was laundered, treated to retard linting, and sealed in plastic bags by a commercial clean-room laundry. The sterile socks were prepared in the laboratory. The subject wore clean undergarments after a daily shower with germicidal detergent (pHisohex*) approximately 8 hours before each examination. On the first and last 4 days of study, the subject was examined while wearing personal clothing ((3) above). The other 16 examinations were carried out while alternately dressed as in (1) and (2) above.

*Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U. S. Department of Health, Education, and Welfare.

TABLE 1

Survival of Naturally Shed Microorganisms from Humans on Stainless-Steel Surfaces Held at 50 C and 40% R.H.

Holding Time in Hours	Average Number of Organisms per Square Foot							
	Test Number							
	1	2	3	4	5	6	7	8
0	3,190	4,330	13,500	9,580	12,500	22,100	24,574	42,720
0.5	655	---	5,700	---	---	---	---	---
1.0	880	1,580	4,770	---	---	---	---	---
1.5	---	1,690	6,640	---	---	---	---	---
4.0	749	986	5,580	---	---	---	---	---
24	662	302	792	2,280	1,530	---	353	1,922
30	---	---	---	2,650	---	---	---	---
48	---	---	---	2,690	---	---	---	---
72	---	---	---	1,550	230	389	---	---
96	---	---	---	410	---	---	---	---
120	---	---	---	---	---	396	---	---
144	---	---	---	---	166	---	---	---
168	---	---	---	---	110	---	151	166
192	---	---	---	---	---	420	---	---
240	---	---	---	---	200	---	---	---
336	---	---	---	---	---	---	94	216
504	---	---	---	---	---	---	22	108
672	---	---	---	---	---	---	65	151

Prior to each 30-minute examination, the R.H. in the microbiotank was reduced to approximately 25% at 66 F. Under the static conditions during examination, the R.H. increased steadily to a maximum of about 85%. The average temperature at the end of the 30-minute examination period was about 68 F. Results of these studies are shown in table 2.

Significant reduction of microorganisms shed while wearing the "bunny suit" was not apparent from these few examinations. Additional examinations are in progress for subsequent statistical analysis.

TABLE 2

Shedding of Viable Particles by a Subject Dressed in Street Clothing and in Two Types of Clean-Room Clothing

Evaluation Number	Microbial Particles Shed per Minute		
	Bunny Suit, Hood, & Sterile Socks	Clean-Room Coat, Cap, Sterile Socks, & Trousers	Personal Clothing & Sterile Socks
1	163	373	867
2	393	383	713
3	373	206	763
4	203	470	509
5	616	477	13,000
6	233	363	15,300
7	413	250	1,560
8	183	736	1,060

Exposure of Stainless-Steel Strips to the Intramural Air of a Hospital Operating Room -- Studies and analyses of the data have been completed in an experiment designed to measure the accumulation of microorganisms on stainless-steel strips exposed to the intramural air of a hospital operating room (Fifth Quarterly Report).

Materials and methods used throughout the study were as follows: Stainless-steel strips 1 x 2 inches in size were washed with hot tap water and detergent, followed by rinses in distilled water, isopropyl alcohol, and ether. After air drying, 80 strips were arranged in a metal tray, wrapped with aluminum foil, and dry-heat sterilized at 170 C for 3 hours. The sterile tray set-up was placed on a Mayo stand in one corner of an operating room in a hospital in Savannah, Georgia. Six strips were aseptically removed at random for microbiological examination immediately after placing the tray and then weekly or biweekly for a total of 21 weeks. The strips were collected in separate, sterile, rubber-stoppered bottles for transport to the laboratory and biological assay within one hour after collection.

Fifty ml of sterile 1% peptone water was added to each bottle. The bottles were mechanically shaken for 5 minutes on a Kahn shaker at 270 strokes per minute. Five-ml portions of each sample were then placed into each of 4 Petri plates and mixed with melted trypticase soy agar. For detection of spores, the remaining broth suspensions were heat shocked at 80 C for 15 minutes and plated as described above. Two of the 4 plates from each procedure were incubated aerobically and 2 from each procedure anaerobically, for 72 hours at 32 C. Anaerobic conditions were achieved in Brewer anaerobic jars using the BBL "GasPak" procedure. Atmospheric conditions were determined in each jar by including plates inoculated with Clostridium sporogenes and Pseudomonas alcoligenes. Colonies were counted using an electronic colony counter. Results of counts obtained during the study are shown in table 3.

The unusually high counts for strips examined at weeks 3 and 4 were discussed in the Fifth Quarterly Report. After the 3rd week, gross particles of dust and lint were observed on the strips. Care was taken not to disturb these particles during collection of the strips. On the 7th week, an increase of aerobic molds was noted. Levels of mold contamination are shown in table 4.

Germicidal Activity of Ethylene Oxide -- Studies on the activity of gaseous ethylene oxide against spores of Bacillus globigii on dust particles were continued utilizing a static exposure chamber. Results of earlier observations using a static system showed variations in the reduction of these spores on glass surfaces when exposed to ethylene oxide (Fourth Quarterly Report). More accurate and sensitive controls to eliminate variations in temperature and relative humidity in the test chamber have been procured to replace the equipment recommended and provided by the manufacturer of the chamber. The newer equipment has been installed and calibrated, and it provides adequate sensitivity and accuracy. A gas

TABLE 3

Levels of Microbial Contamination on Stainless-Steel Strips Exposed to Intramural Air in a Hospital Operating Room

Exposure Time in Weeks	Number of Microorganisms Recovered per Square Foot			
	Unheated (Vegetative Cells)		Heated 80 C 15 Min. (Spores)	
	Aerobic	Anaerobic	Aerobic	Anaerobic
1	$9.7x10^3$	$1.9x10^3$	$9.3x10^1$	0
2	$1.9x10^4$	$3.6x10^3$	$1.6x10^3$	$2.2x10^2$
3	$4.8x10^6$	$5.5x10^6$	$1.8x10^2$	$1.2x10^2$
4	$5.5x10^6$	$9.0x10^6$	$2.9x10^2$	$1.4x10^2$
6	$4.4x10^3$	$1.1x10^4$	$7.2x10^2$	$2.9x10^2$
7	$3.0x10^4$	$1.0x10^4$	$2.4x10^3$	$2.2x10^2$
9	$5.3x10^4$	$1.7x10^4$	$2.2x10^3$	$5.8x10^2$
11	$3.2x10^4$	$1.4x10^4$	$4.7x10^3$	$7.2x10^2$
13	$6.0x10^4$	$2.6x10^4$	$4.3x10^3$	$4.9x10^3$
15	$3.5x10^4$	$1.0x10^4$	$7.2x10^2$	$1.4x10^3$
17	$2.8x10^4$	$1.9x10^4$	$5.0x10^3$	$5.8x10^3$
19	$3.4x10^4$	$1.9x10^4$	$4.3x10^3$	$2.2x10^3$
21	$3.8x10^4$	$2.4x10^4$	$1.4x10^3$	$7.2x10^2$

TABLE 4

Levels of Mold Contamination on Stainless-Steel Strips Exposed to Intramural Air in a Hospital Operating Room

Exposure Time in Weeks	Number of Molds Recovered per Square Foot
7	$1.8x10^3$
9	$2.1x10^3$
11	$2.3x10^3$
13	$1.4x10^3$
15	$1.7x10^3$
17	$1.1x10^3$
19	$2.9x10^3$
21	$2.1x10^3$

chromatograph has been acquired and calibrated for analysis of gas concentrations during test exposures. Methods and procedures for preparation of spores, seeding of institutional dust, nebulization of spore-laden dust onto glass slides, and assay for spores after exposure to ethylene oxide followed those previously used and described (Third Quarterly Report). Exposure of the non-moisture-conditioned spores to ethylene oxide was carried out at approximately 120 F $\pm$ 0.5 F, with an initial humidity of 50% $\pm$ 1.0%. Exposure times were varied from 1 to 6 hours. Various gas concentrations were obtained in the chamber using a mixture of 12% ethylene oxide and 88% freon 12 at differing input pressures. Exposure times to the gas mixture were controlled accurately by rapid evacuation of the exposure chamber. Results are shown in table 5.

The reasons for failure to achieve sterility during some exposure periods are not apparent at this time. Controlled moisture preconditioning of the spore-laden dust prior to exposure to ethylene-oxide gas may enhance its lethal activity. Gas concentrations in the chamber, though relatively constant during each test, varied for 390 to 607 mg/liter during the test series. Reproducible levels were not obtained by adjusting pressures within the exposure chamber. An undesirable fractionation of the gas mixture apparently occurred at some point between the storage cylinder and the sterilizing chamber, causing excessive back-pressure. A method for preventing the back-pressure caused by heating the gas mixture during delivery, will be evaluated. With control of all requisite experimental factors, the biological variations of susceptibility to ethylene-oxide gas can be determined.

TABLE 5

Effect of Ethylene Oxide at 120 F on Dust Particles Laden with B. globigii Exposed for Varying Time Periods

Test Number	Exposure Time (Hours)	Weight of Gas Charge (Pounds)	Pressure of Gas Charge (in. Hg. absolute)	Concentration of Ethylene-Oxide Gas (mg/liter)	Relative Humidity (Percent)		Number of Slides Exposed	Mean Number of *B. globigii* Particles Exposed/Ft^2	Number of Slides Yielding Growth	Mean Remaining Number of Viable Particles/Ft^2
					Start	Finish				
84	6	3.2	30	390	50	40	10	4.4×10^4	1	9.6×10^1
85	5	3.0	30	405	50	40	10	7.2×10^4	0	0
86	4	3.7	30	435	50	50	10	8.3×10^4	0	0
87	2	3.1	30	390	50	40	10	8.4×10^4	0	0
88	3	3.4	30	420	50	50	10	2.0×10^5	0	0
89	1	3.1	30	390	50	50	10	1.2×10^5	0	0
90	6	4.4	36	502	50	20	10	6.3×10^4	0	0
91	5	3.9	36	480	50	40	10	7.2×10^4	0	0
92	1	4.0	36	445	40	20	10	5.8×10^4	0	0
93	4	4.0	36	490	50	40	10	9.0×10^4	1	2.0×10^3
94	2	3.9	36	500	40	25	10	3.7×10^4	1	4.8×10^1
95	3	3.9	36	470	50	25	10	1.3×10^5	1	4.8×10^1
96	6	4.3	40	565	50	31	10	4.6×10^4	2	9.6×10^1
97	5	4.4	40	480	50	34	10	7.6×10^4	0	0
98	4	4.6	40	567	50	35	10	4.2×10^4	0	0
99	1	4.3	40	605	50	41	10	7.3×10^4	0	0
101	3	4.6	40	550	50	37	10	6.5×10^4	0	0
102	2	4.1	40	535	50	46	10	5.7×10^4	0	0

Assembly of the dynamic ethylene-oxide gas chamber is nearly complete. After calibration, studies in parallel with the static chamber are planned.

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